Category Formation for the Mutagenicity of Compounds Acting by Michael-Type Addition and S_NAr Mechanisms

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Introduction

• Identification of toxic modes of action (MOA) of chemicals is essential for risk assessment. Compounds with reactive chemical structures often exhibit significant toxicity.

• Read-across methods may be useful for toxicological predictions within a given mechanism of action for a particular endpoint. It assumes that similarity in structure implies similarity in activity.

• Soft electrophiles react preferentially with thiol groups in proteins and peptides. They fall into the reaction mechanistic domains summarised in Figure 1.

Mechanistic domain	Protein binding reaction	Modified protein
Michael acceptor (M/A esters)		
Double or triple bond with electron-withdrawing substituent X, e.g. –CHO, -COR, -CO_2R, -CN, -SO_2R, -NO_2 etc		
S_NAr electrophiles (chloronitrobenzenes)		

X=halogen or pseudohalogen, Y = ≥ 2 electron withdrawing groups e.g. –NO_2, -CN, -CHO, -CF_3, -SOMe, SO_2Me, ring fused nitrogen

Figure 1. Reaction mechanistic applicability domains

Aims

• In this study the toxicity and reactivity of electrophiles including methacrylate and acrylate esters (M/A esters) and chloronitrobenzenes for different endpoints were investigated in an attempt to form robust categories and allow for read across.

Methods

• **Methacrylate and Acrylate (M/A) esters** (Table 1) were selected on the basis of their structural features, which included alpha-methyl substitution, alcohol chain length, and number of functional groups.

CAS	NAME	Apparent 2nd-order rate constant [liter^{-1}min^{-1}]	log Kow [mol.L_o^{-1} mol$^{-1}L_w$]	Mouse Oral LD50 [mg/kg]	Rat Oral LD50 [mg/kg]	Rabbit Skin LD50 [mg/kg]	LLNA EC3 [%]	Mutagenicity TA100 +S9 (-S9)	*T. pyriformis* Log(1/IGC50) [mol/l]
96-33-3	Methyl acrylate	52	0.7278	827	277	1243	-	Non-mut.	3.547
140-88-5	Ethyl acrylate	26.6	1.2189	1799	800	500	28	Non-mut.	3.516
141-32-2	Butyl acrylate	38.7	2.2011	5880	900	2000	-	Non-mut.	3.519
17831-71-9	Tetraethyleneglycol	143	0.291	-	813	3000	-	Non-mut.	-
80-62-6	Methyl methacrylate	0.325	1.2751	3625	7872	5000	-	Non-mut.	1.782
97-63-2	Ethyl methacrylate	0.139	1.7662	7836	14800	-	-	Non-mut.	2.065
97-88-1	Butylmethacrylate	No appreciable	2.7484	12900	16000	11300	-	Non-mut.	2.731
109-17-1	Tetraethyleneglycol dimethacrylate	1.45	1.3856	-	-	3000	-	Non-mut.	-
97-90-5	Ethyleneglycol dimethacrylate	0.83	2.2088	2000	3300	-	28	Non-mut.	-

Table 1 showing apparent second-order rate constants for the reaction with glutathione and toxicity for different endpoints[2,3]

• **Chloronitrobenzenes** (Table 2) were selected on the basis of their structural relationship to 1-chloro-2,4-dinitrobenzene (CDNB) and spanned the full range of reactivity

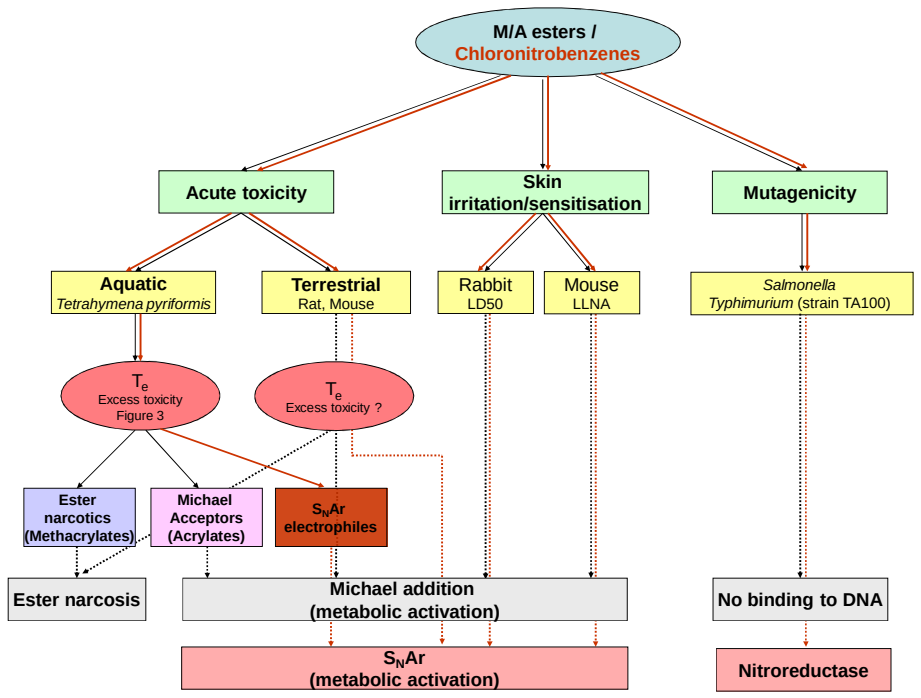
CAS	NAME	GSH [mM]	Relative rate [%]	logKow [mol.L_o^{-1} mol$^{-1}L_w$]	Mouse Oral LD50 [mg/kg]	Rat Oral LD50 [mg/kg]	Rabbit Skin LD50 [mg/kg]	LLNA EC3 [%]	Mutagenicity TA100 +S9 (-S9)	*T. pyriformis* Log(1/IGC 50) [mol/l]
100-00-5	1-chloro-4-nitrobenzene	10	0.1	2.4552	440	420	3040	-	mut	3.43
99-54-7	3,4-dichloronitrobenzene	5	0.32	3.0997	1384	953	200	-	mut	4.16
121-17-5	4-chloro-a,a,a-trifluoro-3-nitrotoluene	5	5.5	3.4182	400	1075	-	-		-
97-00-7	Dinitrochlorobenzene	1	100	2.2729	-	640	130	0,05	mut	5.16
	Ethyl 4-chloro-3,5-dinitrobenzoate	0.5	1590	2.5992	-	-	-	-		-
1930-72-9	Benzonitrile,4-chloro-3,5-dinitro	0.5	72600	1.8199	-	-	-	-		5.66

Table 2 showing GSH reactivity (absolute and relative to CDNB), logKow and toxicity for different endpoints[1,2]

• Prediction of mechanism of action was based on qualitative read across i.e. the identification of a chemical substructure that is common to the two substances (structural analogues) and the assumption that mechanism of action for a test substance can be inferred from the mechanism of action of its analogue

• Excess acute toxicity to *Tetrahymen pyriformis* was established

Results & Discussions



• Figure 2 showing mechanisms of action for M/A esters and chloronitrobenzenes for acute toxicity, skin irritation/sensitisation and mutagenicity.

• **Acute Toxicity:** M/A esters may act by a nonspecific (narcosis) mechanism and as Michael acceptors. Second order rate constants show methacrylates have lower reactivity than acrylates. Figure 3 shows that the majority of compounds exhibiting excess toxicity are associated with direct-acting electrophilic mechanisms of action. For M/A esters, toxicity of methacrylate compounds is consistent with ester narcosis whereas acrylate esters exhibit excess toxicity and may act as Michael acceptors. Rat and mouse oral LD50 values show methacrylates are not toxic to mice and rat, whereas acrylates are shown to be toxic. Oral toxicity does not show a linear relationship with partition coefficient because equilibrium cannot be achieved, hence kinetic effects dominate thermodynamic effects[4]. Metabolism of alpha,beta-unsaturated esters has been shown to occur by hydrolysis and conjugation with GSH. Toxicity in rodents can be organ specific hence differences in chemical reactivity alone is insufficient to explain differences in potency.

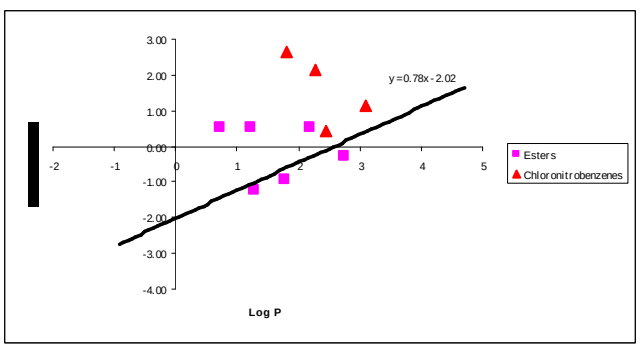


Figure 3. Toxicity to *T. pyriformis* versus log P indicating baseline effect

• Chloronitrobenzenes exhibit toxicity above the baseline effect possibly due to the substituents present in the ortho- and para- positions, they act as S_NAr electrophiles. These compounds show different toxicities in rat and mouse oral LD50s. Differences in reactivity can be explained by the number, strength and position of electron-withdrawing substituents. A different route for metabolic activation is possible.

• **Skin irritation/sensitisation:** M/A esters act as irritants in rabbit skin assay and two have been shown to exhibit skin sensitisation in the mouse LLNA assay. Substituents on the alpha- and beta-carbon atoms can have a significant influence on Michael reactivity. Electron-donating substituents such as methyl groups reduce reactivity. Hence methacrylates are much weaker (or non) sensitizers, compared to acrylates.

• **Mutagenicity:** Acrylate esters were not mutagenic to Salmonella typhimurium in the presence or absence of an exogenous metabolic system, i.e. they did not bind productively to DNA. Chloronitrobenzenes showed mutagenic activity in the *Salmonella typhimurium* (TA100 -S9/+S9) strains, possibly due to bacterial activation i.e. nitroreductase activity, forming hydroxylamine derivatives.

Conclusions

• Soft electrophiles such as acrylates react preferentially with thiol groups in proteins and peptides by Michael addition; methacrylates act by ester narcosis. M/A esters demonstrated acute toxic and skin irritation/sensitisation effects but did not elicit mutagenicity, suggesting no productive binding to DNA.

• Chloronitrobenzenes are weak electrophiles, which react with thiol groups by the S_NAr mechanism. They also demonstrate acute toxicity and skin irritation/sensitisation but additionally may induce mutagenesis via nitroreductase.

Acknowledgment

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References

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